PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 31/535, 31/495, 31/445, 31/40, 31/135

A1

(11) International Publication Number: **WO 99/65495**

(43) International Publication Date: 23 December 1999 (23.12.99)

(21) International Application Number: PCT/US99/13897

(22) International Filing Date: 18 June 1999 (18.06.99)

(30) Priority Data:
60/090,016 19 June 1998 (19.06.98) US

(71) Applicant *(for all designated States except US)*: SMITHKLINE BEECHAM CORPORATION [US/US]; One Franklin Plaza, Philadelphia, PA 19103 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: CALLAHAN, James, F. [US/US]; 8214 Jeanes Street, Philadelphia, PA 19111 (US). CHABOT–FLETCHER, Marie, C. [US/US]; 902 Gay Street, Phoenixville, PA 19460 (US).

(74) Agents: STERCHO, Yuriy, P. et al.; SmithKline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406–0939 (US).

(81) Designated States: AE, AL, AU, BA, BB, BG, BR, CA, CN, CZ, EE, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: INHIBITORS OF TRANSCRIPTION FACTOR NF–κB

(57) Abstract

The present invention provides amino–indanone inhibitors of transcription factor NF–κB and pharmaceutically acceptable salts, hydrates and solvates thereof, pharmaceutical compositions of such compounds, and methods for treating diseases in which activation of NF–κB is implicated. More specifically, the present invention provides methods of treatment of a variety of diseases associated with NF–κB activation including inflammatory disorders; particularly rheumatoid arthritis, inflammatory bowel disease, and asthma; dermatosis, including psoriasis and acute dermatosis; autoimmune diseases; tissue and organ rejection; Alzheimer's disease; stroke; atherosclerosis; restenosis; cancer, including Hodgkins disease; and certain viral infections, including AIDS; osteoarthritis; osteoporosis; and Ataxia Telangiestasia by administering to a patient in need thereof a compound of the present invention.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INHIBITORS OF TRANSCRIPTION FACTOR NF-κB

FIELD OF THE INVENTION

This invention relates in general to methods of inhibiting transcription factor NF-κB using amino-indanones. Such compounds are particularly useful for treating diseases in which activation of NF-κB is implicated. More specifically, these compounds inhibit IκB phosphorylation and subsequent degradation. Such compounds are useful in the treatment of a variety of diseases associated with NF-κB activation including inflammatory disorders; particularly rheumatoid arthritis, inflammatory bowel disease, and asthma; dermatosis, including psoriasis and atopic dermatitis; autoimmune diseases; tissue and organ rejection; Alzheimer's disease; stroke; atherosclerosis; restenosis; cancer, including Hodgkins disease; and certain viral infections, including AIDS; osteoarthritis; osteoporosis; and Ataxia Telangiestasia.

BACKGROUND OF THE INVENTION

Recent advances in scientific understanding of the mediators involved in acute and chronic inflammatory diseases and cancer have led to new strategies in the search for effective therapeutics. Traditional approaches include direct target intervention such as the use of specific antibodies, receptor antagonists, or enzyme inhibitors. Recent breakthroughs in the elucidation of regulatory mechanisms involved in the transcription and translation of a variety of mediators have led to increased interest in therapeutic approaches directed at the level of gene transcription.

NF-κB belongs to a family of closely related dimeric transcription factor complexes composed of various combinations of the Rel/ NF-κB family of polypeptides. The family consists of five individual gene products in mammals, RelA (p65), NF-κB1 (p50/ p105), NF-κB2 (p49/ p100), c-Rel, and RelB, all of which can form hetero- or homodimers. These proteins share a highly homologous 300 amino acid "Rel homology domain" which contains the DNA binding and dimerization domains. At the extreme C-terminus of the Rel homology domain is a nuclear translocation sequence important in the transport of NF-κB from the cytoplasm to the nucleus. In addition, p65 and cRel possess potent transactivation domains at their C-terminal ends.

The activity of NF-κB is regulated by its interaction with a member of the inhibitor IκB family of proteins. This interaction effectively blocks the nuclear localization sequence on the NF-κB proteins, thus preventing migration of the dimer to the nucleus. A wide variety of stimuli activate NF-κB through what are likely to be multiple signal transduction pathways. Included are bacterial products (LPS), some viruses (HIV-1, HTLV-1), inflammatory cytokines (TNFκ, IL-1), and environmental stress. Apparently common to all stimuli however, is the phosphorylation and subsequent degradation of IκB. IκB is phosphorylated on two N-terminal serines by the recently identified IκB kinases (IKK-α and IKK-β). Site-directed mutagenesis studies indicate that these phosphorylations are critical for the subsequent activation of NF-κB in that once phosphorylated the protein is flagged for degradation via the ubiquitin-proteasome pathway. Free from IκB, the active NF-κB complexes are able to translocate to the nucleus where they bind in a selective manner to preferred gene-specific enhancer sequences. Included in the genes regulated by NF-κB are a number of cytokines, cell adhesion molecules, and acute phase proteins.

It is well-known that NF-κB plays a key role in the regulated expression of a large number of pro-inflammatory mediators including cytokines such as IL-6 and IL-8, cell adhesion molecules, such as ICAM and VCAM, and inducible nitric oxide synthase (iNOS). Such mediators are known to play a role in the recruitment of leukocytes at sites of inflammation and in the case of iNOS, may lead to organ destruction in some inflammatory and autoimmune diseases.

The importance of NF-κB in inflammatory disorders is further strengthened by studies of airway inflammation including asthma, in which NF-κB has been shown to be activated. This activation may underlie the increased cytokine production and leukocyte infiltration characteristic of these disorders. In addition, inhaled steroids are known to reduce airway hyperresponsiveness and suppress the inflammatory response in asthmatic airways. In light of the recent findings with regard to glucocorticoid inhibition of NF-κB, one may speculate that these effects are mediated through an inhibition of NF-κB.

Further evidence for a role of NF-κB in inflammatory disorders comes from studies of rheumatoid synovium. Although NF-κB is normally present as an inactive cytoplasmic complex, recent immunohistochemical studies have indicated that NF-κB is present in the nuclei, and hence active, in the cells comprising rheumatoid synovium. Furthermore, NF-

κB has been shown to be activated in human synovial cells in response to stimulation with TNF-κ. Such a distribution may be the underlying mechanism for the increased cytokine and eicosanoid production characteristic of this tissue. *See* Roshak, A. K., et al., *J. Biol. Chem.*, **271,** 31496-31501 (1996).

The NF-κB/Rel and IκB proteins are also likely to play a key role in neoplastic transformation. Family members are associated with cell transformation *in vitro* and *in vivo* as a result of overexpression, gene amplification . gene rearrangements or translocations. In addition, rearrangement and/or amplification of the genes encoding these proteins are seen in 20-25% of certain human lymphoid tumors. In addition, a role for NF-κB in the regulation of apoptosis has been reported stregthening the role of this transcription factor in the control of cell proliferation.

Several NF-κB inhibitors are described in C. Wahl, et al. *J. Clin. Invest.* 101(5), 1163-1174 (1998); R. W. Sullivan, et al. *J. Med. Chem.* 41, 413-419 (1998); J. W. Pierce, et al. *J. Biol. Chem.* 272, 21096-21103 (1997).

The marine natural product hymenialdisine is known to inhibit NF-κB. Roshak, A., et al., *JPET*, **283,** 955-961 (1997). Breton, J. J and Chabot-Fletcher, M. C., *JPET*, **282,** 459-466 (1997).

Amino-indanones are known compounds. General preparation of amino-indanone analogs was described by G. Maury, E.-M. Wu, N. H. Cromwell, ***J. Org. Chem.*** 1968, *33*, 1900-1907. The synthesis of the 3-bromo intermediate was described by B. D. Pearson, R. P. Ayer, N. H. Cromwell, ***J. Org. Chem.*** 1962, *27*, 3038-3044. The synthesis of 2-benzalindanone was desbribed by A. Hassner, N. H. Cromwell, ***J. Org. Chem*** 1958, *80*, 893-900.

We have now discovered a novel method of inhibiting the activation of transcription factor NF-κB using amino-indanones.

SUMMARY OF THE INVENTION

An object of the present invention is to provide a method for treating diseases which may be therapeutically modified by altering the activity of transcription factor NF-κB.

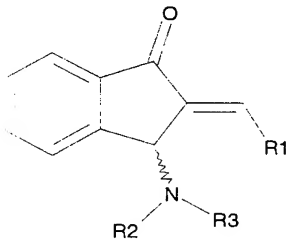
Accordingly, in the first aspect, this invention provides a pharmaceutical composition comprising a compound according to Formula I and a pharmaceutically acceptable carrier, diluent or excipient.

In still another aspect, this invention provides a method of treating diseases in which the disease pathology may be therapeutically modified by inhibiting NF-κB.

In a particular aspect, this invention provides methods for treating a variety of diseases associated with NF-κB activation including inflammatory disorders; particularly rheumatoid arthritis, inflammatory bowel disease, and asthma; dermatosis, including psoriasis and atopic dermatitis; autoimmune diseases; tissue and organ rejection; Alzheimer's disease; stroke; atherosclerosis; restenosis; cancer, including Hodgkins disease; and certain viral infections, including AIDS; osteoarthritis; osteoporosis; and Ataxia Telangiestasia.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method of treatment of diseases associated with NF-κB activation, comprising administering to an animal, particularly a mammal, most particularly a human in need thereof a compound of Formula I:



I

wherein:

R_1 is aryl;

R_2 is selected from the group consisting of: H, C_{1-6}alkyl and aryl;

R_3 is selected from the group consisting of: C_{1-6}alkyl and C_{3-8} cycloalkyl; and

R_2 and R_3 may be joined together to form a heterocyclic ring of 5-7 atoms selected from the group consisting of: C, N, O and S

and pharmaceutically acceptable salts, hydrates and solvates thereof.

The present invention particularly provides methods for treating inflammatory disorders; particularly rheumatoid arthritis, inflammatory bowel disease, and asthma;

dermatosis, including psoriasis and atopic dermatitis; autoimmune diseases; tissue and organ rejection; Alzheimer's disease; stroke; atherosclerosis; restenosis; cancer, including Hodgkins disease; and certain viral infections, including AIDS; osteoarthritis; osteoporosis; and Ataxia Telangiestasia.

Compounds of Formula I selected from the following group are preferred for use in the methods of the present invention:

3-[(N-Butyl)amino]-2-benzalindanone;

3-[(N-cyclohexyl)amino]-2-benzalindanone;

3-Morpholinyl-2-benzalindanone; and

3-Piperidinyl-2-benzalindanone.

Definitions

The present invention includes the use of all hydrates, solvates, complexes and prodrugs of the compounds of this invention. Prodrugs are any covalently bonded compounds which release the active parent drug according to Formula I *in vivo*. If a chiral center or another form of an isomeric center is present in a compound of the present invention, all forms of such isomer or isomers, including enantiomers and diastereomers, are intended to be covered herein. Compounds containing a chiral center may be used as a racemic mixture, an enantiomerically enriched mixture, or the racemic mixture may be separated using well-known techniques and an individual enantiomer may be used alone. In cases in which compounds have unsaturated carbon-carbon double bonds, both the cis (Z) and trans (E) isomers are within the scope of this invention. In cases wherein compounds may exist in tautomeric forms, such as keto-enol tautomers, each tautomeric form is contemplated as being included within this invention whether existing in equilibrium or predominantly in one form.

The meaning of any substituent at any one occurrence in Formula I or any subformula thereof is independent of its meaning, or any other substituent's meaning, at any other occurrence, unless specified otherwise.

"C_{1-6}alkyl" as applied herein is meant to include substituted and unsubstituted methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl and t-butyl, pentyl, n-pentyl, isopentyl, neopentyl and hexyl and the simple aliphatic isomers thereof. Any C_{1-6}alkyl group may be optionally substituted independently by one or two halogens, SR', OR', $N(R')_2$, $C(O)N(R')_2$, carbamyl or C_{1-4}alkyl, where R' is C_{1-6}alkyl.

"C_{3-8} cycloalkyl" as applied herein is meant to include substituted and unsubstituted cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl and the simple aliphatic isomers thereof. Any C_{3-8} cycloalkyl group may be optionally substituted independently by one or two halogens, SR', OR', $N(R')_2$, $C(O)N(R')_2$, carbamyl or C_{1-4}alkyl, where R' is C_{1-6}alkyl.

"Halogen" means F, Cl, Br, and I.

"Ar" or "aryl" means phenyl or naphthyl, optionally independently substituted by one or more of Ph-C_{0-6}alkyl, Het-C_{0-6}alkyl, C_{1-6}alkyl, C_{1-6}alkoxy, Ph-C_{0-6}alkoxy, Het-C_{0-6}alkoxy, OH, CN, CO_2R', or halogen. C_0alkyl means that no alkyl group is present in the moiety. Thus, Ar-C_0alkyl is equivalent to Ar. Two C_{1-6}alkyl groups may be combined to form a 5-7 membered ring, saturated or unsaturated, fused onto the Ar ring. Ph may be optionally substituted with one or more of C_{1-6}alkyl, C_{1-6}alkoxy, OH, CO_2R', or halogen.

As used herein "Het" or "heterocyclic" represents a stable 5- to 7-membered monocyclic ring, which is either saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of N, O and S, and wherein the nitrogen and sulfur heteroatoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized, and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The heterocyclic ring may be attached at any heteroatom or carbon atom which results in the creation of a stable structure, and may optionally be substituted with one or two moieties selected from the group consisting of Ph-C_{0-6}alkyl, Het-C_{0-6} alkyl, C_{1-6}alkyl, C_{1-6}alkoxy, Ph-C_{0-6}alkoxy, Het-C_{0-6}alkoxy, OH, or CN. Two C_{1-6}alkyl groups may be combined to form a 5-7 membered ring, saturated or unsaturated, fused onto the Het ring. Ph may be optionally substituted with one or more of C_{1-6}alkyl, C_{1-6}alkoxy, OH, CO_2R', or halogen. Examples of such heterocycles include the piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolodinyl, 2-oxoazepinyl, azepinyl, pyrrolyl, 4-piperidonyl, pyrrolidinyl, pyrazolyl, pyrazolidinyl, imidazolyl, pyridyl, pyrazinyl, oxazolidinyl, oxazolinyl, oxazolyl, isoxazolyl, morpholinyl, thiazolidinyl, thiazolinyl, thiazolyl, quinuclidinyl, indolyl, quinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, benzoxazolyl, furyl, pyranyl, tetrahydrofuryl, tetrahydropyranyl, thienyl, benzoxazolyl, thiamorpholinyl sulfoxide, thiamorpholinyl sulfone, and oxadiazolyl rings.

Methods of Preparation

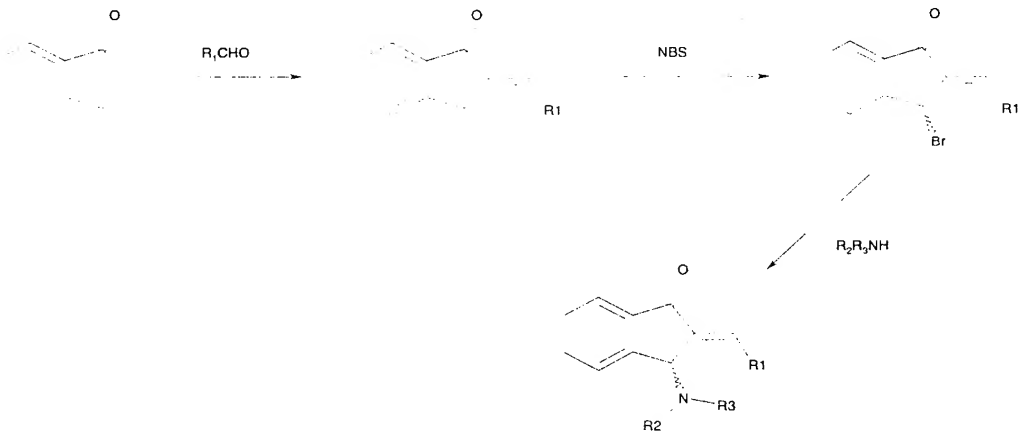
The compounds used in the methods of the present invention may be conveniently prepared by the methods set forth in Scheme 1 below.

General preparation of amino indanone analogs was described by G. Maury, E.-M. Wu, N. H. Cromwell, ***J. Org. Chem.*** 1968, *33*, 1900-1907. The synthesis of the 3-bromo intermediate was described by B. D. Pearson, R. P. Ayer, N. H. Cromwell, ***J. Org. Chem.*** 1962, *27*, 3038-3044. The synthesis of 2-benzalindanone was desbribed by A. Hassner, N. H. Cromwell, ***J. Org. Chem*** 1958, *80*, 893-900.

General Preparation:

The general preparation is shown in Scheme 1. An indanone is treated with a benzylaldehyde in base to give a 2-benzalindanone. Bromination of a 2-benzalindanone with NBS in CCl_4 gives a 3-bromo-2-benzalindanone. Treatment of a 3-bromo-2-benzalindanone with an alkyl primary or secondary amine in benzene gives a 3-alkylamino-2-benzalindanone.

Scheme 1



Referring to the methods of preparing the compounds of Formula I set forth in Schemes 1 above, the skilled artisan will appreciate that the present invention includes all novel intermediates required to make the compounds of Formula I.

The starting materials used herein are commercially available or are prepared by routine methods well known to those of ordinary skill in the art and can be found in standard reference books, such as the COMPENDIUM OF ORGANIC SYNTHETIC METHODS, Vol. I-VI (published by Wiley-Interscience).

Acid addition salts of the compounds of Formula I are prepared in a standard manner in a suitable solvent from the parent compound and an excess of an acid, such as hydrochloric, hydrobromic, hydrofluoric, sulfuric, phosphoric, acetic, trifluoroacetic, maleic, succinic or methanesulfonic. Certain of the compounds form inner salts or zwitterions which may be acceptable. Cationic salts are prepared by treating the parent compound with an excess of an alkaline reagent, such as a hydroxide, carbonate or alkoxide, containing the appropriate cation; or with an appropriate organic amine. Cations such as Li^+, Na^+, K^+, Ca^{++}, Mg^{++} and NH_4^+ are specific examples of cations present in pharmaceutically acceptable salts. Halides, sulfate, phosphate, alkanoates (such as acetate and trifluoroacetate), benzoates, and sulfonates (such as mesylate) are examples of anions present in pharmaceutically acceptable salts.

This invention provides a pharmaceutical composition which comprises a compound according to Formula I and a pharmaceutically acceptable carrier, diluent or excipient. Accordingly, the compounds of Formula I may be used in the manufacture of a medicament. Pharmaceutical compositions of the compounds of Formula I prepared as hereinbefore described may be formulated as solutions or lyophilized powders for parenteral administration. Powders may be reconstituted by addition of a suitable diluent or other pharmaceutically acceptable carrier prior to use. The liquid formulation may be a buffered, isotonic, aqueous solution. Examples of suitable diluents are normal isotonic saline solution, standard 5% dextrose in water or buffered sodium or ammonium acetate solution. Such formulation is especially suitable for parenteral administration, but may also be used for oral administration or contained in a metered dose inhaler or nebulizer for insufflation. It may be desirable to add excipients such as polyvinylpyrrolidone, gelatin, hydroxy cellulose, acacia, polyethylene glycol, mannitol, sodium chloride or sodium citrate.

Alternately, these compounds may be encapsulated, tableted or prepared in an emulsion or syrup for oral administration. Pharmaceutically acceptable solid or liquid carriers may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Solid carriers include starch, lactose, calcium sulfate dihydrate, terra alba,

magnesium stearate or stearic acid, talc, pectin, acacia, agar or gelatin. Liquid carriers include syrup, peanut oil, olive oil, saline and water. The carrier may also include a sustained release material such as glyceryl monostearate or glyceryl distearate, alone or with a wax. The amount of solid carrier varies but, preferably, will be between about 20 mg to about 1 g per dosage unit. The pharmaceutical preparations are made following the conventional techniques of pharmacy involving milling, mixing, granulating, and compressing, when necessary, for tablet forms; or milling, mixing and filling for hard gelatin capsule forms. When a liquid carrier is used, the preparation will be in the form of a syrup, elixir, emulsion or an aqueous or non-aqueous suspension. Such a liquid formulation may be administered directly p.o. or filled into a soft gelatin capsule.

For rectal administration, the compounds of Formula I may also be combined with excipients such as cocoa butter, glycerin, gelatin or polyethylene glycols and molded into a suppository.

The methods of the present invention include topical administration of the compounds of Formula I. By topical administration is meant non-systemic administration, including the application of a compound of the invention externally to the epidermis, to the buccal cavity and instillation of such a compound into the ear, eye and nose, wherein the compound does not significantly enter the blood stream. By systemic administration is meant oral, intravenous, intraperitoneal and intramuscular administration. The amount of a compound of Formula I (hereinafter referred to as the active ingredient) required for therapeutic or prophylactic effect upon topical administration will, of course, vary with the compound chosen, the nature and severity of the condition being treated and the animal undergoing treatment, and is ultimately at the discretion of the physician.

While it is possible for an active ingredient to be administered alone as the raw chemical, it is preferable to present it as a pharmaceutical formulation. The active ingredient may comprise, for topical administration, from 0.01 to 5.0 wt%.of the formulation.

The topical formulations of the present invention, both for veterinary and for human medical use, comprise an active ingredient together with one or more acceptable carriers therefor, and optionally any other therapeutic ingredients. The carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of where treatment is required such as: liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving the active ingredient in a suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 90-100 C for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy basis. The basis may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol or macrogols. The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surface active agent such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic

materials such as silicaceous silicas, and other ingredients such as lanolin, may also be included.

Utility of the Present Invention

The compounds of Formula I are useful as inhibitors of NF-κB. The present invention provides useful compositions and formulations of said compounds, including pharmaceutical compositions and formulations of said compounds.

The present invention also provides methods of treatment of diseases associated with NF-κB activation, which methods comprise administering to an animal, particularly a mammal, most particularly a human in need thereof a compound of Formula I. The present invention particularly provides methods for treating inflammatory disorders; particularly rheumatoid arthritis, inflammatory bowel disease, and asthma; dermatosis, including psoriasis and atopic dermatitis; autoimmune diseases; tissue and organ rejection; Alzheimer's disease; stroke; atherosclerosis; restenosis; cancer, including Hodgkins disease; and certain viral infections, including AIDS; osteoarthritis; osteoporosis; and Ataxia Telangiestasia.

For acute therapy, parenteral administration of a compound of Formula I is preferred. An intravenous infusion of the compound in 5% dextrose in water or normal saline, or a similar formulation with suitable excipients, is most effective, although an intramuscular bolus injection is also useful. Typically, the parenteral dose will be about 0.01 to about 50 mg/kg; preferably between 0.1 and 20 mg/kg, in a manner to maintain the concentration of drug in the plasma at a concentration effective to inhibit activation of NF-κB. The compounds are administered one to four times daily at a level to achieve a total daily dose of about 0.4 to about 80 mg/kg/day. The precise amount of an inventive compound which is therapeutically effective, and the route by which such compound is best administered, is readily determined by one of ordinary skill in the art by comparing the blood level of the agent to the concentration required to have a therapeutic effect.

The compounds of Formula I may also be administered orally to the patient, in a manner such that the concentration of drug is sufficient to inhibit NF-κB or to achieve any other therapeutic indication as disclosed herein. Typically, a pharmaceutical composition containing the compound is administered at an oral dose of between about 0.1 to about 50 mg/kg in a manner consistent with the condition of the patient. Preferably the oral dose would be about 0.5 to about 20 mg/kg.

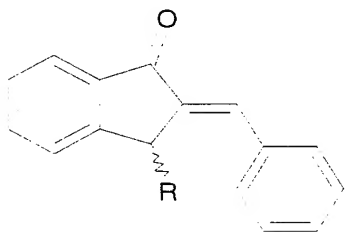
The compounds of Formula I may also be administered topically to the patient, in a manner such that the concentration of drug is sufficient to inhibit NF-κB or to achieve any other therapeutic indication as disclosed herein. Typically, a pharmaceutical composition containing the compound is administered in a topical formulation of between about 0.01% to about 5% w/w.

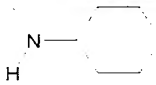
No unacceptable toxicological effects are expected when compounds of the present invention are administered in accordance with the present invention.

The ability of the compounds described herein to inhibit the activation of NF-κB is clearly evidenced in their ability to inibit NF-κB-driven reporter gene activity (*see* Table 1). The utility of the present NF-κB inhibitors in the therapy of diseases is premised on the importance of NF-κB activation in a variety of diseases.

Table 1

Inhibition of NF-κB-driven Reporter Gene Activity



Compound Number	R	Reporter Assay IC_{50} uM
1		2.2, 1.0
2		1.7
3		1.9
4		3.0
5		14% @ 5 uM
6	H	inactive

NF-κB plays a key role in the regulated expression of a large number of pro-inflammatory mediators including cytokines such as IL-6 and IL-8 (Mukaida *et al.*, 1990; Liberman and Baltimore, 1990; Matsusaka *et al.*, 1993), cell adhesion molecules, such as ICAM and VCAM (Marui *et al.*, 1993; Kawai *et al.*, 1995; Ledebur and Parks, 1995), and inducible nitric oxide synthase (iNOS) (Xie *et al.*, 1994; Adcock *et al.*, 1994). (Full reference citations are at the end of this section). Such mediators are known to play a role in the recruitment of leukocytes at sites of inflammation and in the case of iNOS, may lead

to organ destruction in some inflammatory and autoimmune diseases (McCartney-Francis *et al.*, 1993; Kleemann *et al.*, 1993). Importantly, the compounds described herein inhibit IL-8 synthesis and the production of nitric oxide, a product of iNOS activity (*see* Table 2).

TABLE 2

Anti-inflammatory Activity of Compound 2 in Table 1

In vitro	
IL-1-induced PGE2 production in RSF	IC_{50} = 1 uM
In vivo	
Phorbol-ester-induced ear inflammation	
Ear swelling	85% inhibition @ 1 mg/ear
Inflammatory cell infiltration	29% inhbiition @ 1 mg/ear

Evidence for an important role of NF-κB in inflammatory disorders is obtained in studies of asthmatic patients. Bronchial biopsies taken from mild atopic asthmatics show significant increases in the number of cells in the submucosa staining for activated NF-κB, total NF-κB, and NF-κB-regulated cytokines such as GM-CSF and TNFα compared to biopsies from normal non-atopic controls (Wilson *et al.*, 1998). Furthermore, the percentage of vessels expressing NF-κB immunoreactivity is increased as is IL-8 immunoreactivity in the epithelium of the biopsy specimens (Wilson *et al.*, 1998). As such, inhibition of IL-8 production through the inhibition of NF-κB, as has been demonstrated by these compounds would be predicted be beneficial in airway inflammation.

Recent studies suggest that NF-κB may also play a critical role in the pathogenesis of inflammatory bowel disease (IBD). Activated NF-κB is seen in colonic biopsy specimens from Chron's disease and ulcerative colitis patients (Ardite *et al.*, 1998; Rogler *et al.*, 1998; Schreiber *et al.*, 1998). Activation is evident in the inflamed mucosa but not in uninflamed mucosa (Ardite *et al.*, 1998; Rogler *et al.*, 1998) and is associated with increased IL-8 mRNA expression in the same sites (Ardite *et al.*, 1998). Furthermore, corticosteroid treatment strongly inhibits intestinal NF-κB activation and reduces colonic

inflammation (Ardite *et al.*, 1998; Schreiber *et al.*, 1998). Again, inhibition of IL-8 production through the inhibition of NF-κB, as has been demonstrated by these compounds would be predicted be beneficial in inflammatory bowel disease.

Animal models of gastrointestinal inflammation provide further support for NF-κB as a key regulator of colonic inflammation. Increased NF-κB activity is observed in the lamina propria macrophages in 2,4,6,-trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice with p65 being a major component of the activated complexes (Neurath *et al.*, 1996; Neurath and Pettersson, 1997). Local administration of p65 antisense abrogates the signs of established colitis in the treated animals with no signs of toxicity (Neurath *et al.*, 1996; Neurath and Pettersson, 1997). As such, one would predict that small molecule inhibitors of NF-κB would be useful in the treatment of IBD.

Further evidence for a role of NF-κB in inflammatory disorders comes from studies of rheumatoid synovium. Although NF-κB is normally present as an inactive cytoplasmic complex, recent immunohistochemical studies have indicated that NF-κB is present in the nuclei, and hence active, in the cells comprising human rheumatoid synovium (Handel *et al.*, 1995; Marok *et al.*, 1996; Sioud *et al.*, 1998) and in animal models of the disease (Tsao *et al.*, 1997). The staining is associated with type A synoviocytes and vascular endothelium (Marok *et al.*, 1996). Furthermore, constitutive activation of NF-κB is seen in cultured synoviocytes (Roshak *et al.*, 1996; Miyazawa *et al.*, 1998) and in synovial cell cultures stimulated with IL-1β or TNFα (Roshak *et al.*, 1996; Fujisawa *et al.*, 1996; Roshak *et al.*, 1997). Thus, the activation of NF-κB may underlie the increased cytokine production and leukocyte infiltration characteristic of inflamed synovium. The ability of these compounds to inhibit NF-κB and thereby inhibit the production of eicosanoids by these cells would be predicted to yield benefit in rheumatoid arthritis (*see* Table 2).

Mukaida N, MaheY, Matsushima K (1990) Cooperative interaction of nuclear factor-κB- and cis-regulatory enhancer binding protein-like factor binding elements in activating the interleukin-8 gene by pro-inflammatory cytokines. *J Biol Chem* 265: 21128-21133

Liberman TA, Baltimore D (1990) Activation of interleukin-6 gene expression through NF-κB transcription factor. *Mol Cell Biol* 10: 2327-2334

Matsusaka T, Fujikawa K, Nishio Y, Mukaida N, Matsushima K, Kishimoto T, Akira S (1993) Transcription factors NF-IL6 and NF-κB synergistically activate transcription of the inflammatory cytokines interleukin 6 and interleukin 8. *Proc Natl Acad Sci USA* 90: 10193-10197

Marui N, Offerman MK, Swerlick, R, Kunsch C, Rosen CA, Ahmad M, Alexander RW, Medford RM (1993) Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. *J Clin Invest* 92: 1866-1874

Kawai M, Nishikomori R, Jung E-Y, Tai G, Yamanak C, Mayumi M, Heike T (1995) Pyrrolidine dithiocarbamate inhibits intercellular adhesion molecule-1 biosynthesis induced by cytokines in human fibroblasts. *J Immunol* 154: 2333-2341

Ledebur HC, Parks TP (1995) Transcriptional regulation of the intracellular adhesion molecule-1 gene by inflammatory cytokines in human endothelial cells. *J Biol Chem* 270: 933-943

Xie Q, Kashiwabara Y, Nathan C (1994) Role of transcription factor NF-κB/Rel in induction of nitric oxide synthase. *J Biol Chem* 269: 4705-4708

Adcock IM, Brown CR, Kwon O, Barnes PJ (1994) Oxidative stress induces NF-κB DNA binding and inducible NOS mRNA in human epithelial cells. *Biochem Biophys Res Commun* 199: 1518-1524

McCartney-Francis N, Allen JB, Mizel DE, Albina JE, Xie Q, Nathan CF, Wahl SM (1993) Suppression of arthritis by an inhibitor of nitric oxide synthase. *J Exp Med* 178: 749-754

Kleemann R, Rothe H, Kolb-Bachofen V, Xie Q, Nathan C, Martin S, Kolb H (1993) Transcription and translation of inducible nitric oxide synthase in the pancreas of prediabetic BB rats. *FEBS Lett* 328: 9-12

Wilson SJ, Wallin A, Sandstrom T, Howarth PH, Holgate ST (1998) The expression of NF-kappa-B and associated adhesion molecules in mild asthmatics and normal controls. *J Allergy Clin Immunol* 101: 616

Ardite E, Panes J, Miranda M, Salas A, Elizalde JI, Sans M, Arce Y, Bordas JM, Fernandez-Checa JC, Pique JM (1998) Effects of steroid treatment on activation of nuclear factor κB in patients with inflammatory bowel disease. *Br J Pharmacol* 124: 431-433

Rogler G, Brand K, Vogl D, Page S, Hofmeister R, Andus T, Knuechel R, Baeuerle PA, Scholmerich J, Gross V (1998) Nuclear factor κB is activated in macrophage and epithelial cells of inflamed intestinal mucosa. *Gastroenterol* 115: 357-369

Schreiber S, Nikolaus S, Hampe J (1998) Activation of nuclear factor κB in inflammatory bowel disease. *Gut* 42: 477-484

Neurath MF, Pettersson S, Meyer zum Buschenfelde K-H, Strober W (1996) Local administration of antisense phosphorothioate oligonucleotides to the p65 subunit of NF-κB abrogates established experimental colitis in mice. *Nature Med* 2: 998-1004

Neurath MF, Pettersson S (1997) Predominant role of NF-κB p65 in the pathogenesis of chronic intestinal inflammation. *Immunobiol* 198: 91-98

Handel ML, McMorrow LB, Gravallese EM (1995) Nuclear factor -κB in rheumatoid synovium; localization of p50 and p65. *Arthritis Rheumatism* 38: 1762-1770

Marok R, Winyard PG, Coumbe A, Kus ML, Gaffney K, Blades S, Mapp PI, Morris CJ, Blake DR, Kaltschmidt C, Baeuerle PA (1996) Activation of the transcription factor nuclear factor-κB in human inflamed synovial tissue. *Arthritis Rheumatism* 39: 583-591

Sioud M, Mellbye O, Forre O (1998) Analysis of the NF-κB p65 subunit, Fas antigen, Fas ligand and Bcl-2-related proteins in the synovium of RA and polyarticular JRA. *Clin ExpRheumatol* 16: 125-134

Tsao PW, Suzuki T, Totsuka R, Murata T, Takagi T, Ohmachi Y, Fujimura H, Takata I (1997) The effect of dexamethasone on the expression of activated NF-κB in adjuvant arthritis. *Clin Immunol Immunopathol* 83: 173-178

Roshak AK, Jackson JR, McGough K, Chabot-Fletcher M, Mochan E, Marshall L (1996) Manipulation of distinct NFκB proteins alters interleukin-1β-induced human rheumatoid synovial fibroblast prostaglandin E2 formation. *J Biol Chem* 271: 31496-31501

Miyazawa K, Mori A, Yamamoto K, Okudaira H (1998) Constitutive transcription of the human interleukin-6 gene by rheumatoid synoviocytes; spontaneous activation of NF-κB and CBF1. *Am J Pathol* 152, 793-803

Fujisawa K, Aono H, Hasunuma T, Yamamoto K, Mita S, Nishiola K (1996) Activation of transcription factor NF-κB in human synovial cells in response to tumor necrosis factor α. *Arthritis Rheumatism* 39: 197-203

Roshak AK, Jackson JR, Chabot-Fletcher M, Marshall L (1997) Inhibition of NFκB-mediated interleukin-1β-stimulated prostaglandin E2 formation by the marine natural product hymenialdisine. *J Pharmacol Exp Therapeut* 283: 955-961

Biological Assays

The compounds of this invention may be tested in one of several biological assays to determine the concentration of compound which is required to have a given pharmacological effect.

Assays of NF-κB activity are conducted using a cell based luciferase reporter assay as described in Breton, J. J and Chabot-Fletcher, M. C., *JPET*, **282,** 459-466 (1997). Briefly, U937 human histiocytic lymphoma cell line permanently transfected with the NF-κB reporter plasmids (see below) are cultured in the above medium with the addition of 250 μg/ml Geneticin (G418 sulfate, Life Technologies, Grand Island, NY). The luciferase reporter assay is conducted in the transfected U937 clones. These are twice centrifuged at 300 xg for 5 min and resuspended in RPMI 1640 with 10% FBS to a density of 1 x 10^6 cells/ml. One ml aliquots are added to the wells of 24-well plates. Compound or dimethyl sulfoxide (DMSO) carrier (1 μl) is added to the appropriate wells and the plates are incubated at 37°C, 5% CO_2 for 30 min. The stimulus is added (5 ng/ml TNFκ, 100 ng/ml LPS, or 0.1 μM PMA) and the samples incubated for 5 hours at 37°C, 5% CO_2, transferred to 1.9 ml polypropylene tubes, and centrifuged at 200 xg for 5 min. The cell pellets are washed twice in 1 ml PBS without Ca^{2+} and Mg^{2+}, and centrifuged as indicated above. The resulting cell pellets are lysed in 50 μl 1x lysis buffer (Promega Corporation, Madison, WI), vortexed and incubated for 15 min at room temperature. A 20 μl aliquot of each lysate is transferred to an opaque white 96-well plate (Wallac Inc., Gaithersburg, MD) and assayed for luciferase production in a MicroLumat LB 96 P luminometer (EG&G Berthold, Bad Wilbad, Germany). The luminometer dispenses 100 μl luciferase assay reagent (Promega

Corporation, Madison, WI) into each well and the integrated light output is recorded for 20 sec. Light output is measured in relative light units (RLUs).

NF-κB activity may also be measured in an electrophoretic mobility shift assay (EMSA) to assess the presence of NF-κB protein in the nucleus. The cells of interest are cultured to a density of $1x10^6$ /ml. The cells are harvested by centrifugation, washed in PBS without Ca^{2+} and Mg^{2+} and resuspended in PBS with Ca^{2+} and Mg^{2+} at $1x10^7$ cells/ml. To examine the effect of compound on the activation of NF-κB, the cell suspensions are treated with various concentrations of drug or vehicle (DMSO, 0.1%) for 30 min at 37°C prior to stimulation with TNFα (5.0ng/ml) for an additional 15 min. Cellular and nuclear extracts are prepared follows Briefly, at the end of the incubation period the cells ($1x10^7$ cells) are washed 2x in PBS without Ca^{2+} and Mg^{2+}. The resulting cell pellets are resuspended in 20 µl of Buffer A (10mM Hepes (pH 7.9), 10mM KCl, 1.5mM $MgCl_2$, 0.5mM dithiothreitol (DTT) and 0.1% NP-40) and incubated on ice for 10 min. The nuclei are pelleted by microcentrifugation at 3500 rpm for 10 min at 4°C. The resulting supernatant was collected as the cellular extract and the nuclear pellet was resuspended in 15 µl Buffer C (20mM Hepes (pH 7.9), 0.42M NaCl, 1.5mM $MgCl_2$, 25% glycerol, 0.2mM EDTA, 0.5mM DTT, and 0.5mM phenylmethylsulphonyl fluoride (PMSF)). The suspensions are mixed gently for 20 min at 4°C then microcentrifuged at 14,000 rpm for 10 min at 4°C. The supernatant is collected and diluted to 60 µl with Buffer D (20mM Hepes (pH 7.9), 50mM KCl, 20% glycerol, 0.2mM EDTA, 0.5mM DTT, and 0.5mM PMSF). All samples are stored at -80°C until analyzed. The protein concentration of the extracts is determined according to the method of Bradford (Bradford, 1976) with BioRad reagents.

The effect of compounds on transcription factor activation is assessed in an electrophoretic mobility shift assay (EMSA) using nuclear extracts from treated cells as described above. The double stranded NF-κB consensus oligonucleotides (5'-AGTTGAGGGGACTTTCCCAGGC-3') are labelled with T_4 polynucleotide kinase and [g-^{32}P]ATP. The binding mixture (25 µl) contains 10mM Hepes-NaOH (pH 7.9), 4mM Tris-HCl (pH 7.9), 60mM KCl, 1mM EDTA, 1mM dithiothreitol, 10% glycerol, 0.3 mg/ml bovine serum albumin, and 1 µg poly(dI-dC)•poly(dI-dC). The binding mixtures (10 µg nuclear extract protein) are incubated for 20 min at room temperature with 0.5 ng of ^{32}P-labelled oligonucleotide (50,000-100,000 cpm) in the presence or absence of unlabeled competitor after which the mixture is loaded on a 4% polyacrylamide gel prepared in 1X

Tris borate/EDTA and electrophoresed at 200 V for 2 h. Following electrophoresis the gels are dried and exposed to film for detection of the binding reaction.

The effect of compounds on the phosphorylation of IκB may be monitored in a Western blot. Cellular extracts are subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10% gels (BioRad, Hercules, CA) and the proteins transferred to nitrocellulose sheets (Hybond™-ECL, Amersham Corp., Arlington Heights, IL). Immunoblot assays are performed using a polyclonal rabbit antibody directed against IκBα or IκBβ followed with a peroxidase-conjugated donkey anti-rabbit secondary antibody (Amersham Corp., Arlington Heights, IL). Immunoreactive bands are detected using the Enchanced Chemiluminescence (ECL) assay system (Amersham Corp., Arlington Heights, IL).

Effects on eicosanoid production by human synovial fibroblasts (RSF) are assessed using primary cultures of human RSF. These are obtained by enzymatic digestion of synovium obtained from adult patients with rheumatoid. Cells are cultured in Earl's Minimal Essential Medium (EMEM) containing 10% fetal bovine serum (FBS), 100 units/ml penicillin and 100 µg/ml streptomycin (GIBCO, Grand Island, NY), at 37°C and 5% CO_2. Cultures are used at passages 4 through 9 in order to obtain a more uniform type I fibroblast population. For some studies, fibroblasts are plated at 5 x 10^4 cells/ml in 16mm (diameter) 24 well plates (Costar, Cambridge, MA). Cells are exposed to an optimal dose of IL-1ß (1ng/ml; Roshak *et al.* 1996a)(Genzyme, Cambridge, MA) for the designated time. Drugs in DMSO vehicle (1%) are added to the cell cultures 15 minutes prior to the addition of IL-1. Prostaglandin E_2 levels in cell-free medium collected at the termination of the culture period are directly measured using enzyme immunoassay (EIA) kits purchased from Cayman Chemical Co. (Ann Arbor, MI). Sample or standard dilutions are made with experimental medium.

Anti-inflammatory activity in vivo is assessed using the phorbol ester-induced ear inflammation model in mice. Phorbol myristate acetate (PMA) (4µg/20µl acetone) is applied to the inner and outer surfaces of the left ear of Male Balb/c mice (6/group) (Charles River Breeding Laboratories, Wilmington, MA). Four hours later, compound dissolved in 25µl acetone is applied to the same ear. The thickness of both ears is measured with a dial micrometer (Mitutoyo, Japan) after 20 hours and a second topical dose of compound is applied. Twenty-four hours later, ear thickness measurements are taken and the data expressed as the change in thickness (x 10^{-3}cm) between treated and

untreated ears. The inflamed left ears are then removed and stored at -70°until assayed for myeloperoxidase (MPO) activity, a measure of inflammatory cell infiltration.

Inflammatory cell infiltration is assessed through the measurement of myeloperoxidase activity present in the inflammed ear tissue. Partially thawed ear tissues are minced and then homogenized (10% w/v) with a Tissumizer homogenizer (Tekmar Co., Cincinnati, OH) in 50 mM phosphate buffer (pH 6) containing 0.5% HTAB. The tissue homogenates are taken through three cycles of freeze-thaw, followed by brief sonication (10s). MPO activity in the homogenates is determined as follows. The appearance of colored product from the MPO-dependent reaction of o-dianisidine (0.167 mg/ml, Sigma Chemical, St. Louis, MO) and hydrogen peroxide (0.0005%) is measured spectrophotometrically at 460 nm. Supernatant MPO activity is quantified kinetically (change in absorbance measured over 3 min, sampled at 15 s intervals) using a Beckman DU-7 spectrophotometer and a kinetics analysis package (Beckman Instruments, Inc., Sommerset, NJ). One unit of MPO activity is defined as that degrading one micromole of peroxide per minute at 25°C.

Effects on inflammation-mediated cartilage breakdown is measured in an in vitro cartilage explant system. In this model bovine articular cartilage explants are incubated for 4 days/96 hours with or without rHuIL-1 alpha to stimulate cartilage breakdown in the presence or absence of test compound. The supernatants are removed for the nitric oxide assays. Nitric oxide was measured using the Greiss reaction and read spectrophotometrically at 530nm. This reaction measures nitrite (NO_2) which is the stable end product of nitric oxide.

General

Nuclear magnetic resonance spectra were recorded at either 250, 300 or 400 MHz using, respectively, a Bruker AM 250, Bruker, Bruker ARX 300 or Bruker AC 400 spectrometer. $CDCl_3$ is deuteriochloroform, DMSO-d_6 is hexadeuteriodimethylsulfoxide, and CD_3OD is tetradeuteriomethanol. Chemical shifts are reported in parts per million (d) downfield from the internal standard tetramethylsilane. Abbreviations for NMR data are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, app = apparent, br = broad. J indicates the NMR coupling constant measured in Hertz. Continuous wave infrared (IR) spectra were recorded on a

Perkin-Elmer 683 infrared spectrometer, and Fourier transform infrared (FTIR) spectra were recorded on a Nicolet Impact 400 D infrared spectrometer. IR and FTIR spectra were recorded in transmission mode, and band positions are reported in inverse wavenumbers (cm^{-1}). Mass spectra were taken on either VG 70 FE, PE Syx API III, or VG ZAB HF instruments, using fast atom bombardment (FAB) or electrospray (ES) ionization techniques. Elemental analyses were obtained using a Perkin-Elmer 240C elemental analyzer. Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. All temperatures are reported in degrees Celsius.

Analtech Silica Gel GF and E. Merck Silica Gel 60 F-254 thin layer plates were used for thin layer chromatography. Both flash and gravity chromatography were carried out on E. Merck Kieselgel 60 (230-400 mesh) silica gel.

Where indicated, certain of the materials were purchased from the Aldrich Chemical Co., Milwaukee, Wisconsin.

Examples

In the following synthetic examples, temperature is in degrees Centigrade (°C). Unless otherwise indicated, all of the starting materials were obtained from commercial sources. Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. These Examples are given to illustrate the invention, not to limit its scope. Reference is made to the claims for what is reserved to the inventors hereunder.

Example 1

Preparation of 3-[(N-Buyl)amino]-2-benzalindanone.

a) 2-Benzalindanone.

Following the procedure of A. Hassner, N. H. Cromwell, ***J. Org. Chem*** 1958, *80*, 893-900, benzaldehyde (6.05 mL, 53.6 mmol) was added to a solution of indanone (Aldrich, 7.08 g, 53.6 mmol) in ethanol and KOH (600 mg, 10.6 mmol) at 0 C and the reaction sat in the refrigerator overnight. The reaction mixture was filtered, washed with 50% aqueous EtOH and then recrystallized from hot ethanol to give 5.84 g of 2-

benzalindanone. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93(d, 1H, J = 7.7), 7.75-7.35 (m, 8H), 4.08 (s, 2H).

b) 3-Bromo-2-benzalindanone.

Following the procedure of B. D. Pearson, R. P. Ayer, N. H. Cromwell, ***J. Org. Chem***. 1962, *27*, 3038-3044, compound (1.0 g, 4.55 mmol) from Example 1(a) in CCl_4 (15 mL) was treated with NBS (810 mg, 4.55 mmol) and benzoyl peroxide (50 mg) and the mixture heated at reflux with a heat lamp for 1 h. The reaction was cooled, filtered and the filtrate evaporated to give 3-bromo-2-benzalindanone which was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.1- 7.2 (m, 10H), 6.40 (s, 2H).

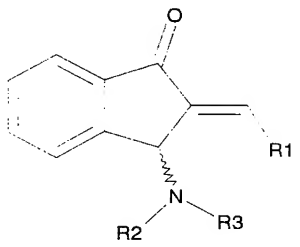
c) 3-[(N-Buyl)amino]-2-benzalindanone

Following the procedure of G. Maury, E.-M. Wu, N. H. Cromwell, ***J. Org. Chem***. 1968, *33*, 1900-1907, compound from Example 1(b) in benzene was treated with n-butylamine (300 uL, 3.04 mmol) and the solution stirred at RT for 24 h. The reaction was evaporated and the residue purified by flash chromatography (silica gel, 10% ethyl acetate in hexane) to give 353 mg of 3-[(N-butyl)amino]-2-benzalindanone. A portion of the free base in ether was treated with 1N HCl in ether to give the hydrochloride salt as a solid. ES-MS (M+H)+ m/e 292; ^{1}H NMR (300 MHz, $CDCl_3$) δδ8.2 (d, 1 H, J = 7.0 Hz), 8.03 (s, 1H), 7.98 (d, 1H, J = 6 Hz), 7.84 (t, 1H, J = 6 Hz), 7.75- 7.50 (m, 7H), 6.7 (br s, 1H)2.48 (br s1H), 2.28 (br s, 1H), 1.6-1.3 (m, 2H), 0.95-0.8 (m, 2H), 0.65 (t, 3H, J=6.5 Hz).

The above specification and Example fully disclose how to make and use the compounds of the present invention. However, the present invention is not limited to the particular embodiments described hereinabove, but includes all modifications thereof within the scope of the following claims. The various references to journals, patents and other publications which are cited herein comprise the state of the art and are incorporated herein by reference as though fully set forth.

We claim:

1. A pharmaceutical composition comprising a compound of Formula I:



I

wherein:

R_1 is aryl;

R_2 is selected from the group consisting of: H, C_{1-6}alkyl and aryl;

R_3 is selected from the group consisting of: C_{1-6}alkyl and C_{3-8} cycloalkyl; and

R_2 and R_3 may be joined together to form a heterocyclic ring of 5-7 atoms selected from the group consisting of: C, N, O and S;

and a pharmaceutically acceptable carrier, diluent or excipient.

2. A pharmaceutical composition according to Claim 1 wherein R_2 is H.

3. A pharmaceutical composition according to Claim 2 wherein R_3 is selected from the group consisting of: butyl and cyclohexyl.

4. A pharmaceutical composition according to Claim 1 wherein R_2 and R_3 may be joined together to form a heterocyclic ring of 5-7 atoms selected from the group consisting of: C, N, O and S.

5. A pharmaceutical composition according to Claim 4 wherein R_3 is selected from the group consisting of: morpholinyl and piperidinyl.

6. A pharmaceutical composition according to Claim 1 wherein said compound is selected from the group consisting of:

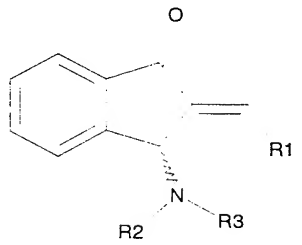
3-[(N-Butyl)amino]-2-benzalindanone;

3-[(N-cyclohexyl)amino]-2-benzalindanone;

3-Morpholinyl-2-benzalindanone; and

3-Piperidinyl-2-benzalindanone.

7. A method of inhibiting NF-κB comprising administering to a patient in need thereof an effective amount of a compound of Formula I:



I

wherein:

R_1 is aryl;

R_2 is selected from the group consisting of: H, C_{1-6}alkyl and aryl;

R_3 is selected from the group consisting of: C_{1-6}alkyl and C_{3-8} cycloalkyl; and

R_2 and R_3 may be joined together to form a heterocyclic ring of 5-7 atoms selected from the group consisting of: C, N, O and S.

8. A method according to Claim 7 wherein said compound is selected from the group consisting of:

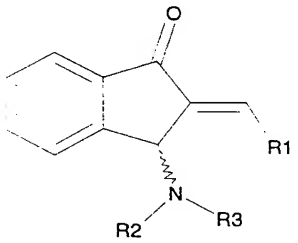
3-[(N-Butyl)amino]-2-benzalindanone;

3-[(N-cyclohexyl)amino]-2-benzalindanone;

3-Morpholinyl-2-benzalindanone; and

3-Piperidinyl-2-benzalindanone.

9. A method of treating a disease characterized by excessive NF-κB activation comprising inhibiting said excessive activation by administering to a patient in need thereof an effective amount of a compound of Formula I:



I

wherein:

R_1 is aryl;

R_2 is selected from the group consisting of: H, C_{1-6}alkyl and aryl;

R_3 is selected from the group consisting of: C_{1-6}alkyl and C_{3-8} cycloalkyl; and

R_2 and R_3 may be joined together to form a heterocyclic ring of 5-7 atoms selected from the group consisting of: C, N, O and S.

and pharmaceutically acceptable salts, hydrates and solvates thereof.

10. A method according to Claim 9 wherein said compound is selected from the group consisting of:

3-[(N-Butyl)amino]-2-benzalindanone;

3-[(N-cyclohexyl)amino]-2-benzalindanone;

3-Morpholinyl-2-benzalindanone; and

3-Piperidinyl-2-benzalindanone.

11. A method according to Claims 9 and 10 wherein said disease is an inflammatory disorder.

12. A method according to Claim 11 wherein said disease is selected from the group consisting of: rheumatoid arthritis, inflammatory bowel disease, and asthma.

13. A method according to Claim 9 and 10 wherein said disease is dermatosis.

14. A method according to Claim 13 wherein said disease is selected from the group consisting of: psoriasis and atopic dermatitis.

15. A method according to Claim 9 and 10 wherein said disease is selected from the group consisting of: autoimmune diseases; tissue and organ rejection; Alzheimer's disease; stroke; atherosclerosis; restenosis; osteoarthritis; osteoporosis; and Ataxia Telangiestasia.

16. A method according to Claim 9 and 10 wherein said disease is cancer.

17. A method according to Claim 16 wherein said cancer is Hodgkins disease.

18. A method according to Claim 9 and 10 wherein said disease is AIDS.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/13897

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/535, 31/495, 31/445, 31/40, 31/135
US CL :514/231.2, 255, 319, 429, 657

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/231.2, 255, 319, 429, 657

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

HCAPLUS- compounds encompassed by the claims in pharmaceutical compositions.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	Database HCAPLUS on STN, American Chemical Society, AN 1981:514971, GUPTA, R.C. et al. 'N-substituted .alpha.-aminoalkylacrylophenones and some related compounds: a new class of spermicidal agents,' abstract, Indian J. Chem., 1981, 20(B), 303-307.	1 and 4-5 ---------- 6
X ---- Y	Database HCAPLUS on STN, American Chemical Society, AN 1969:106237, MAURY, G. et al. 'Mobile keto allyl systems. VIII. Properties of 2-(.alpha.-aminobenzyl)-1-indenones.' abstract, J. Org. Chem., 1969, 34(3), 596-601.	1-3 ------ 6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
27 AUGUST 1999

Date of mailing of the international search report
20 OCT 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
M. MOEZIE
JOYCE BRIDGERS
PARALEGAL SPECIALIST
CHEMICAL MATRIX
Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/13897

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 11-18
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.